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# Study on the use of Artemia salina as bioindicator in the ecotoxicological evaluation of landfill leachate

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## Abstract

Landfill leachate deserves special attention because it presents itself as a potentially polluting liquid that can attack nearby natural resources if it is not carefully treated and disposed of in a controlled manner, or it may be reused in other activities. The present work had the objective of evaluating the toxicity of untreated landfill leachate on the test organism *A. salina*. The physical chemical characterization of the landfill leachate for the presence of metals and salts was performed according to the methodology proposed by the American Public Health Association - APHA (2005). After hatching, about 10 nauples of *A. salina* were transferred to 15 x 150 mm test tubes containing 10 mL (saline water and the landfill leach to be tested). Seven treatments with four replicates were used. The LC5048h of the landfill leachate used in the present study was mathematically obtained in the dose with 28.4% of leachate. The parameters of salinity, ammonia and sulfates are directly related to the value of the LC5048h.

**Keywords**: *Ecotoxicology,Landfill leachete, A. salina.*

## 1. Introduction

From the promulgation of the National Policy on Solid Waste, prevention and reduction in waste generation has become a constant discussion tool among generators and waste receivers. From the environmental point of view, the destination of this waste is the sanitary landfill, however, it presents as environmental liability to the generation of landfill leachate (BRASIL, 2010).

Landfill leachate deserves special attention because it presents itself as a potentially polluting liquid that can attack nearby natural resources if it is not treated and discarded in a

careful, controlled manner or reused in other activities (RENOU et al., 2008; SALEM et al., 2008).

The generation of leachate in the sanitary landfill occurs due to the degradation of solid waste by the presence of microorganisms, most of them bacteria acting on aerobic or anaerobic metabolism, characterized by the existence and the absence of oxygen, respectively; besides these, the conditions of the environment, characteristic of the residues and precipitation contribute to a greater or less volume of percolado (IPT, 1995).

The reuse of the landfill leachate should be based on a chemical and physical characterization that indicates its contaminating potential. However, it is fundamental to evaluate its biological implications and possible interactions (COSTA et al., 2008; KALCIKOVA et al., 2011).

Ecotoxicological analyzes have been used in the monitoring of industrial, domestic and leachate effluents in order to minimize environmental impact, evaluate the efficiency of treatment plants and also as a requirement for obtaining and maintaining permits with environmental agencies. These analyzes aim to investigate the effects of manufactured chemical substances and other anthropogenic or natural materials on aquatic organisms (SOUSA, 2002).

For the analysis of the ecotoxicological effects of "simple" substances or mixtures that may be used in human activities, ecotoxicity tests or bioassays should be carried out to predict the potential impact of a xenobiotic (toxic agent) on the environment (FLOHR et al., 2005).

Toxicity means any adverse effect manifested by test organisms, which may range from genetic changes, immobility, deformities to lethality. It should be noted that the use of ecotoxicological tests in the evaluation of the quality of water and effluents was officially regulated, at federal level, through Resolution no. 357 of March 17, 2005 of the National Environmental Council (CONAMA, 2005).

A *Artemia salina* is a saltwater microcrustacean. It is characterized by a low cost bioassay of toxicity, quick results and non-aseptic techniques. Due to its natural habitat, it represents an important biological indicator for environments with high salt concentrations (CARVALHO et al., 2009). The present work had the objective of evaluating the toxicity of untreated landfill leachate on the test organism *A. salina*.

## 2. Materials and methods

### *2.1 Physicochemical characterization of landfill leachate*

The physicochemical characterization of landfill leachate was held at the Environmental Technology Laboratory of the State University of Rio de Janeiro, Labtam-UERJ.

The landfill leachate used throughout the study was provided by the company Haztec Technology and Environmental Planning SA, manager of the Waste Treatment Center (CTR) located on Highway Engineer Alexandre Drable - Cotiara in the municipality of Barra Mansa - RJ, geographic coordinates 22°35'11.52"S and 44°12'54.31"W.

The physicochemical characterization (Table 1) was performed according to the methodology of the American Public Health Association - APHA (2005), for the presence and concentration of heavy metals and salts. The physico-chemical analysis of the leachate comprised the determination of the following parameters: electrical conductivity (EC), pH, salinity and total dissolved solids (TDS), through direct reading on a multiparameter measuring mark Waterproof - Test PCS R 35; and chemical oxygen demand (COD) using the closed reflux colorimetric method of digestion of the samples with the digester made in block TECNAL TE - - 021 - DRY BLOCH, then reading the samples in a spectrophotometer HACH - DR5000. The determination of cations

and anions was performed by ion chromatography using a DIONEX machine - Model: ICS 3000 - Chromeleon 6.8.

For the determination of the concentrations of metals (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn), samples were digested according to the EPA-3051a procedure: Microwave Assisted Acid Digestion of Sediments, sludges, the Soil sand Oils (USEPA, 1998): 20 mL of sample and 10 ml of nitric acid ($HNO_3$) PA was placed in a teflon bottle which was then closed and heated in a microwave oven (Manufacturer: Milestone model Start E) with a power of 600W for 20 min (heating to 170 °C for 10 min and holding at 170 °C for 10 min). The product obtained was filtered on paper filter and swelled in 100 ml volumetric flasks flat bottom. Samples were read in duplicate, in a spectrometer of atomic absorption flame - FAAS (abbreviation - Flame Atomic Absorption Spectrometer) of vary, Model: ASA 240. The operating conditions were used in FAAS: manual sampling mode; measurement mode: integration, display time: 5s, flame type: Air / acetylene; airflow: 10.0 L $min^{-1}$, acetylene flow: 2.0 L $min^{-1}$.

*2.1.1 Cysts sampling*

To perform the tests were used cysts of *A. salina*. The cysts were from JBL® with 95% of explosion and validity until July/2019, directly, without any previous procedure.

*2.1.2 Preparation of cysts explosion*

The assay was performed according to the procedure described in the literature (MEYER et al., 1982; CETESB, 1991; MCLAUGHLIN et al., 1995) with adaptations. For hatching of the *A. salina* cysts, a saline solution was prepared with 50 g of sea salt + 1 g $NaHCO_3$ to 0.5 $L^{-1}$ of distilled water. The pH of the solution was 8.8. In addition, 1 g of cysts were placed in artemio JBL® and remained for 48 hours until complete hatching, with constant aeration and controlled temperature of 27 to 30 °C.

*2.2 Toxicological bioassay test*

After hatching, approximately 10 nauplii of *A. salina* were transferred to 15 x 150 mm test tubes containing 10 mL (saline water and landfill leach to be tested), at the following leachate dosages: T1 = 3.125% leached of landfill + 96.875% water; T2 = 6.25% landfill leachate + 93.75% water; T3 = 12.5% landfill leachate + 87.5% water; T4 = 25% landfill leachate + 75% water; T5 = 50% landfill leachate + 50% water and T6 = 100% landfill leachate. For the negative control group (TC), 100% of saline water was used. All treatments were performed with four replicates.

*2.2.1 Mortality assessment*

After 48 hours of exposure, live and dead nauplii were counted, being considered alive all those that presented any type of movement when observed near a light source. Because it is an active organism in saline water, the lack of movement and sedimentation are the indicators of death of the same. Only those tests in which the control had a mortality equal to or less than 10% of the population were considered valid. After exposure, the mean percentage and standard deviation of individuals killed according to each dose tested were calculated. In addition, the LC5048h was calculated by determining the function obtained from the correlation between the percentage of dead individuals and the dose of the leachate tested. Subsequently, the value of y was replaced with 0.5 and $LC50_{48h}$ was calculated. The function was determined with 95% reliability.

*2.3 Statistical analysis*

The data were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% probability between them (COSTA NETO, 1977; MILLER; MILLER, 1993).

**3. Results and discussion**

According to the physicochemical parameters shown in Table 1 it is possible to classify the landfill leachate as a recently produced (KURNIAWAN et al., 2010). It is also observed that when comparing the physicochemical parameters of the leachate to the standard of effluent quality for release CONAMA 430/2011, the raw leachate does not have features that allow its disposal in the receiving body, defining it as a pollutant potential. Evaluating physical-chemical characteristics of the raw landfill leachate (Table 1), it is noted that especially the sodium salinity parameters are above the reference value.

Table 1. Physicochemical characterization of landfill leachate

| **Evaluated parameters** | **Unit** | **Observed values** | **CONAMA 430** |
|---|---|---|---|
| | | | **Effluent to disposal[1]** |
| pH (water) | -------- | 9.0 | 5.0 - 9.0 |
| Conductivity | dS $m^{-1}$ | 33.3 | ND |
| COD | mg $L^{-1}$ | 5,592 | 120 |
| Total dissolved solids | mg $L^{-1}$ | 23,600 | ND |
| Salinity | mg $L^{-1}$ | 16,900 | ND |
| Aluminum * | mg $L^{-1}$ | 7,625 | ND |
| Ammonium ** | mg $L^{-1}$ | 595.27 | 20.0 |
| Calcium ** | mg $L^{-1}$ | 95.38 | ND |
| Chloride ** | mg $L^{-1}$ | 857.50 | 250 |
| Iron * | mg $L^{-1}$ | 13.66 | 15.0 |
| Lithium ** | mg $L^{-1}$ | 15.22 | 2.5 |
| Magnesium ** | mg $L^{-1}$ | 15.37 | ND |
| Manganese * | mg $L^{-1}$ | 0.31 | 1.0 |
| Nitrate ** | mg $L^{-1}$ | 496.75 | 10 |
| Potassium ** | mg $L^{-1}$ | 694.46 | ND |
| Sodium** | mg $L^{-1}$ | 1,172.46 | ND |
| Sulfate** | mg $L^{-1}$ | 846.00 | 250 |
| Zinc * | mg $L^{-1}$ | 1.13 | 5.0 |

pH - hydrogenionic potential; COD - Chemical Oxygen Demand; * Reading via atomic absorption; ** Reading via ion chromatograph; ND - not detected; [1]Standard for effluent disposal into receiving waters.

The middle values of the percentage of individuals killed as a function of the different leachate treatments are shown in figure 1. There was an increase in the mortality rate of the individuals as the leachate dose increased. For treatments control group, and treatments groups T1-3,125% and T2-6,25%), no significant differences were observed. From the T3 treatment of the pollutant, a mean mortality rate of 15% was observed. This value increased to 30% in T4 treatment, 75% in T5 treatment and 100% in T6 treatment. The quadratic relation was observed between the leachate dosage and the dead organisms, evidencing the dose-response effect.

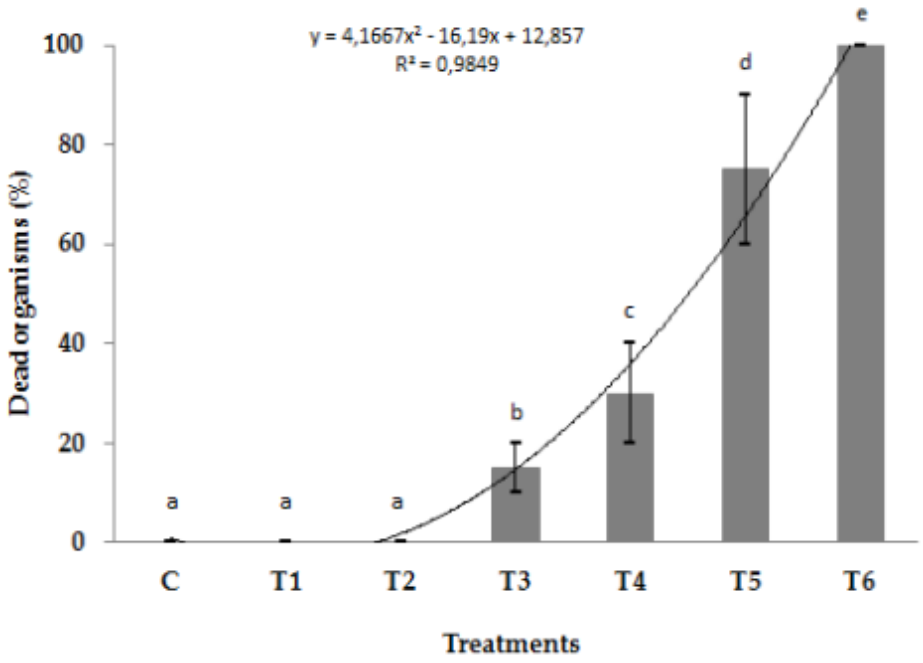


Figure 1: Average percentage of mortality of *A. salina* as a function of different doses of leachate from the landfill. Means followed by the same lowercase letter do not differ statistically from each other by Tukey test ($p<0.05$).

For the calculation of the $LC50_{48h}$ the quadratic polynomial function was described by $y = 4,1667x^2 - 16,19x + 12,857$ with $R^2 = 0,9849$. Using this function and replacing y by 0.5, the dose of 28.4% was found, which represents 2.84 mL of landfill leachate.

Meyer et al. (1982) reports that for different substances, the lower the LC50 value found, the greater the toxic effect of the corresponding substance. The leachate tested showed no toxicity to *A. salina* since the calculated $LC50_{48h}$ was 400 ppm. According to the World Health Organization (WHO), substances with LC50 values below 1000 ppm in *A. salina* (MEYER et al., 1982) are considered to be toxic.

The negative impact produced by the landfill leachate is high. Studies have shown that adverse effects can be observed on the soil, both in the chemical and physical characteristics, as well as on the biota even at distances of more than 100 m from the landfill, as well as changes in the aquatic biota, especially in the vicinity of the discharge (BAUN et al. 2003).

Teixeira (2008) identified that the acute toxicity (LC50) of landfill leachate in *A. salina* occurs at a dosage of 67.77%, suggesting that this is due to the presence of metals such as iron, manganese and lead.

Bortolotto et al. (2009), observed the LC50 occurrence of an untreated leachate on *A. salina* at the dose of 71.63%. The authors also suggest that the toxicity is due to the presence of the metals aluminum, iron, manganese and zinc, in addition to the high concentration of sulfates in solution.

Silva et al. (2004) identified the LC50 for doses with 25.58% and 11.89% of landfill leachate at different collection times, which demonstrates the great heterogeneity of the mixture. The authors suggest that the severe salinity, high concentration of ammonia, presence of sulfates and heavy metals would be the factors that contributed most to the toxicity of the leachate against the test organism. However, the values obtained from the study leachate for the salinity parameters (16,900 ppm) may be directly related to the salinity of the species, result found for LC50, as well as ammonia (595.27 ppm) and sulfates (846.00 ppm).

The characterization of the leachate is very complex because its composition varies according to the age of the landfill and the type of waste deposited, which in turn also depends on the consumption habits of the population (KULIKOWSKA and KLIMIUK, 2008).

## 4. Conclusion

The *A. saline* was sensitive to concentrations of crude landfil above 6.25%. The $LC50_{48h}$ of the landfill leachate used in the present study was obtained mathematically in the dose with 28.4%.

The parameters of salinity, ammonia and sulfates may be directly related to the results found for LC50.

The toxicity test using *A. salina* proves to be an excellent methodology to verify the potential impact of the landfill leachate, being of low cost, easy handling, fast execution and high sensitivity.

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